EFFICIENCY OF WATER UTILIZATION IN CRASSULACEAN ACID METABOLISM PLANTS WHEN IN CAM *VERSUS* OUT OF CAM

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SUMMARY

The assimilation of CO_2 in Crassulacean acid metabolism (CAM) plants occurs mainly in the dark and it is influenced by environment. *Sedum telephoides* and *Kalanchoe daigremontiana* when grown IN CAM had a higher number of stomata and assimilated more CO_2 per given leaf area, which accounts for their higher total growth than plants grown OUT OF CAM. However, plants grown OUT OF CAM with their lower stomatal density were more efficient in water usage, producing twice as much growth per gram of water transpired.

INTRODUCTION

Many desert plants belong to the Crassulaceae family and have distinct morphological features that aid in water conservation resulting in low transpiration rates and high water use efficiencies (Joshi *et al.*, 1965). Most plants in this group open their stomata during dark and close them during the day (Loftfield, 1921; Mathur, 1975; Nishida, 1963; Thompson-Cloudley, 1954). In these plants organic acids accumulate during the night and decrease during the day and this is referred to as Crassulacean acid metabolism (CAM). This process is assumed to have adaptive significance for conservation of water and CO_2 in certain types of succulent plants (Kunitake and Saltman, 1958; Walker, 1966; Laetch, 1974).

Varied environmental conditions can bring some CAM plants either IN CAM (when plant shows diurnal fluctuations of as much as 20 meq/100 g FW of titratable acidity) or OUT OF CAM (when the concentrations of titratable acidity remain low with no diurnal fluctuations). When the plants are grown IN CAM versus OUT OF CAM environments, the rates of transpiration and dry matter accumulation should be different. Therefore, a comparative study was made on photosynthetic rates, transpiration and dry matter accumulation of these plants maintained continuously under IN CAM or OUT OF CAM environments.

MATERIAL AND METHODS

Individual mother plants of *Sedum telephoides* and *Kalanchoe daigremontiana* were selected and propagated to give populations of isogenetic plants. The plants were grown in pots in a (1:1) peat : perlite mixture in the growth chamber either IN CAM

(12 hours of 20 Klx incandescent and fluorescent light at 30° C and 12 hours of dark at 15°C) or OUT OF CAM (24 hours of 20 Klx incandescent and flourescent light at 30°C). All measurements were done on the plants maintained under these environments. Roots were washed and total fresh weight of each of 28 different plants of equal growth of *S. telephoides* and *K. daigremontiana* was determined. Ten plants were used to determine the dry weight and four plants were grown either IN CAM or OUT OF CAM. Since the measurement of the dry weights showed 0.009 and 0.012 percent variance the resulting means of 0.32 and 0.50 respectively, were used to calculate the dry weight of plants at the end of the experiments.

The experimental plants were transferred in plastic containers with 1250 g of sand in each pot and sealed except for cotton around the stem of the plant. The necessary amount of water was added in each pot to bring it up to field capacity according to the procedure outlined by Richard (1951). The plants were grown for 9 weeks under IN CAM or OUT OF CAM. The amount of water added each week was equal to the weekly utilization of water from the individual pot (the difference in weights of container with plants and container with no plant) which kept the pots at field capacity.

The average transpiration rate was recorded at weekly intervals and used to estimate total water loss of each plant during the entire experimental period. Growth during the period was expressed as amount of increase in dry matter obtained by subtracting the original derived dry weights from the final dry weight. The transpiration ratio was obtained by dividing the total grams of water lost by grams of dry matter accumulated.

The rate of net photosynthesis (Pn) of attached leaves of *S. telephoides* was measured after 9 weeks using a Beckman Infrared (model 215A) analyzer using the differential method. Hayaski Denkio automatic leaf area meter was used for leaf area measurement. Each leaf was measured at least five times in different positions to get a mean representative value in square decimeters. Data were converted to mg $CO_2 dm^{-2}$ hr^{+1} according to the following equation :

$$\text{mg } CO_2 dm^{-2} hr^{-1} = \frac{\Delta \mu l/L(\text{ppm})\ CO_2 \times \text{air flow (ml/hr)} \times K}{1 \times 10^6 \times \text{leaf area } (dm^2)}$$

$$\text{where } K = \frac{44{,}000 \text{ mg } CO_2}{22.4} \times \frac{T(\text{abs})}{T + t(\text{obs})}$$

A square chamber of 10×10 cm dimensions made after the air seal principle of Wolf *et al.* (1969) was used to enclose the leaves. The upper and lower halves of the chamber had their spacers removed from one edge to serve as air outlet and to accomodate the leaf. A nylon thread attached to the upper and lower halves of the chamber positioned the leaf midway between the walls of chamber. Two tubes 14 cm long and 1 cm in diameter with 0.2 cm holes were glued to the upper half of chamber. These holes served as air inlet and outlet manifold for the chamber.

Leaves from the top 5 to 7 cm of the test plants were used for the measurement of Pn. Leaf temperature was measured by a thermocouple placed on the leaf and maintained at 30°±2°C. Compressed ambient air of 340 to 360 μl/L (ppm) CO_2 was passed over the leaf at one liter per minute. The leaf chamber was partially sealed

with Plastite adhesive clay to allow excess air eflux and prevent the entry of atmospheric CO_2. Exactly 0.5 litres of sample air and reference air were pumped from the leaf chamber into Dryrite before entering the infrared CO_2 analyzer. A continuous recording of CO_2 concentration was made on a Sargent Recorder.

RESULTS

The data in table I show water utilization of *S. telephoides* and *K. daigremontiana*. With the exception of species, plants in soil held at field capacity had higher transpiration ratios and produced higher dry matter when IN CAM than OUT OF CAM environment (Table I). It was apparent that plants which have CAM type metabolism grow successfully by conserving water in regions where the environmental conditions such as daily temperature and photoperiod change quite frequently.

Table I. Dry matter production and transpiration ratio of plants grown for 9 weeks at constant soil moisture IN CAM and OUT OF CAM

Plant	Total dry matter production (g DW)		Transpiration ratio (g H_2O/g DW)	
	IN CAM	OUT OF CAM	IN CAM	OUT OF CAM
Sedum telephoides	1.52	1.27	73.87	36.16
Kalanchoe daigremontiana	1.84	1.26	65.51	37.93

The data in Table II show that Pn rates of *S. Telephoides* IN CAM versus OUT OF CAM were different. Evidently, Pn reached its maximum value by the middle of the dark period when IN CAM. The rate of Pn at its peak was 5.15 mg $CO_2 dm^{-2}$ hr^{-1}. The Pn in *S. telephoides* when OUT OF CAM did not exceed 1.0 to 1.2 mg CO_2 dm^{-2} hr^{-1}. The stomatal density of CAM plants changed when grown continuously IN CAM or OUT OF CAM environment (Mathur, 1975).

Table II. Net photosynthesis (mg $CO_2/dm^2/hr$) measurement of CAM plants grown for 9 weeks IN CAM and OUT OF CAM in Sedum telephoides

mg $CO_2/dm_3/hr$			
Beginning of light 9 AM	Middle of light 3 PM	Beginning of dark 9 PM	Middle of dark 3 AM
	IN CAM		
0.50	0.60	0.60	5.15
	OUT OF CAM		
1.0	1.1	1.0	1.2

Discussion

In *S. telephoides*, because of contrasting response of CO_2 assimilation in the dark and the behaviour of stomata when IN CAM and OUT OF CAM had differences in transpiration rates and dry matter accumulation (Table I). Similar dark CO_2 assimilation has been reported in other CAM plants (Gregory *et al.*, 1954; Zabka *et al.*, 1958; Zabka and McMahen, 1964; Zabka and Chaturvedi, 1975). In chrysanthemum plants photoperiod alone has been found to regulate stomatal behaviour (Schwabe, 1952). The night time opening of stomates in the present study on CAM plants is attributed to either the formation of organic acids which caused lowering of the pH and subsequently increased the osmotic value or due to swelling of amphoteric colloids as proposed by Nishida (1963). The decarboxylation of organic acids in the light then reversed the process controlling stomatal size and the closure of stomates during the daytime. This reverse stomatal response was found when plants were grown OUT OF CAM thus confirming that major CO_2 assimilation in these test plants occurred in the dark.

The accumulation of dry matter at the end of 9 weeks was also higher when plants were IN CAM than OUT OF CAM, but the efficiency of water utilization was 50 per cent higher when plants were OUT OF CAM (Table I). The OUT OF CAM environment not only caused stomatal closure but induced morphological change by reducing the number of stomata per dm^2 as compared to plants when IN CAM. The low CO_2 assimilation under OUT OF CAM is the most probable cause of lower dry matter production (Table II). The OUT OF CAM environment can be similar to the situation reported by Nishida (1963) where succulent plants did not show CO_2 fixation during the dark.

Therefore, on the basis of the present results, it is concluded that in CAM plants the stomata play an important role in CO_2 assimilation, transpiration and dry matter production in specific environments.

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